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Chemical Composition and Biological Activity of *Alchornea cordifolia* (Schum. and Thonn.) Mull. Arg. (Euphorbiaceae) Leaf

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Abstract

Alchornea cordifolia, commonly known as the Christmas bush, has been traditionally used in West Africa and the Congo against inflammation and infections. This study aimed to evaluate the chemical composition and anti-inflammatory and antibacterial effects of the methanol leaf extract of *A. cordifolia*. Color and precipitation reactions were used to qualitatively analyze the phytochemicals. Proximate analysis of the powdered leaves was performed using the Association of Official Analytical Chemists (AOAC) methods, whereas the heavy metal content was evaluated using atomic absorption spectrophotometry (AAS).
Acute toxicity was evaluated using Lorke's method. The anti-inflammatory properties of the extract were determined using the carrageenan-induced paw edema model, and the disc diffusion method was used to screen the extract against clinical isolates. Phytochemical analysis revealed the presence of glycosides, saponins, flavonoids, phenolic compounds, terpenoids, alkaloids, and steroids. Proximate and mineral analyses showed high moisture content (15.40%) and potassium content (37.40 mg/kg). No mortality was recorded across the doses, although writhing was observed at 5000 mg/kg of the extract. There was a significant percentage inhibition of edema with a prolonged duration of action. The highest percentage inhibition (35.4%) at 800 mg/kg was the best result compared to that of the negative control group and other doses. The extract exhibited significant inhibitory action against *P. aeruginosa* compared to gentamicin. The extract shows potential in inhibiting inflammatory swelling and the growth of bacteria, especially *P. aeruginosa*, thus supporting its use as an anti-inflammatory and anti-infective agent.

Keywords: *Alchornea cordifolia,* antibacterial, proximate analysis, anti-inflammatory, *Pseudomonas*, *Streptococcus*

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Introduction

Both wild and cultivated plants have been used for medicine and food by numerous ethnic groups (Foster and Duke, 2014). The World Health Organization has acknowledged the use of herbs by a large percentage of the human population. In many developing nations, herbal preparations are believed to be safe, readily available, and inexpensive. These advantages come with some challenges, such as the inability to properly monitor its preparation and sales, which has resulted in products that could be injurious to users. Pharmaceutical and chemical studies of herbs have resulted in life-saving drugs such as morphine from opium poppy, aspirin from willow bark, pilocarpine from *Maranham jaborandi*, quinine from cinchona bark, and digoxin from foxglove (Stoen and Moe, 2006). Approximately 200 billion pounds of herbs and spices are produced annually in the United States (Harvey 2008). Different tribes use medicinal plants to reduce pain and treat infections. Medicinal plants have been used as significant therapeutic agents and valuable raw materials for the development of many orthodox and contemporary medicines.

Alchornea cordifolia (Schum. and Thonn.) Mull. Arg. is an evergreen, dioecious, small, woody plant of 8 m in height, belonging to the Euphorbiaceae family, native to tropical Africa, and is widely distributed across the continent (Agyare *et al*., 2016). It is cultivated in Ghana and the Democratic Republic of the Congo for its medicinal and culinary value. Crushed leaves of *A. cordifolia* are traditionally used as a mouth wash to treat mouth ulcers and dental caries, applied on wounds as cicatrizing agents to relieve pain, and used to treat skin infections and diarrhea (Mavar-Manga *et al*., 2007). Previous studies have shown that aqueous, ethanol, and methanol extracts from *A. cordifolia* leaves inhibit Bacillus *subtilis, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Ebi, 2001; Djimeli *et al*., 2017; Adounkpe *et al*., 2022). In addition, the aqueous, methanol, and aqueous-methanol extracts were reported to reduce croton oil and egg albumin-induced inflammation (Osadebe and Okoye, 2003; Manga *et al*., 2004). This study further evaluated the effect of methanol leaf extract on *Streptococcus* mutants and carrageenan-induced paw edema.

Materials and Methods

Collection and identification of sample

Alchornea cordifolia leaves were obtained in October 2024 from Area 4 West Zone, World Bank Housing Estate in Osisioma-Ngwa Local Government Area of Abia State, with GPS location of $5^0$8'5" N, $7^0$20'7" E. The plant was identified and authenticated by Prof. H. A. Akinnibosun of the Herbarium Unit of the Department of Plant Biology and Biotechnology, University of Benin. The specimen number issued was UBH-A502, and a sample of the plant specimen was deposited in the herbarium.

Preparation and extraction of sample

The leaves were air-dried for two (2) weeks and subsequently pulverized into powder using an industrial grinding machine. The powdered sample (200 g) was macerated in 1200 mL (1.2 L) of methanol (99%) with initial agitation at 30 min intervals for 6 h, whereas the total extraction time was 72 h. The mixture was filtered through a size 1 filter paper, and the filtrate obtained was concentrated under vacuum at 50 ^{0}C in a rotary evaporator.

Qualitative phytochemical Screening

The chemical components in *A. cordifolia* powdered leaf were qualitatively examined using color and precipitate formation reactions (Sofowora 1993; Evans 2009). The phytochemicals screened included glycosides, saponins, flavonoids, phenolic compounds, tannins, phytosterols, terpenoids, and alkaloids.

Proximate Analysis

Proximate analysis was performed on the powdered leaves using the methods described in the Association of Official Analytical Chemist (AOAC) manual (1990). The parameters determined included ash, moisture, crude fiber, crude fat, crude protein, and total carbohydrate contents.

Elemental Composition

The method described by Dmitrievich (2013) was used. One gram of the powdered sample was placed in Kjeldahl flasks, and 10 mL of mixed acid (nitric acid and perchloric acid mixture, ratio 3:1) was added to each flask. The flask and its contents were mildly heated for approximately 20 min at 40°C and then increased to 100°C for another 20 min. The sample was allowed to cool, and approximately 20 mL distilled water was added. The mixture was filtered into a 100 mL standard flask and subsequently made up to the mark with distilled water. From this solution, sodium (Na) and potassium (K) were assayed using a Flame Photometer, while magnesium (Mg), calcium (Ca), iron (Fe), and copper (Cu) were assayed using an Atomic Absorption Spectrophotometer.

Experimental animals

Thirty (36) Wistar rats (195-240 g) of both sexes were obtained from the Animal House of the Plant Biology and Biotechnology Department, University of Benin. The

animals were kept in clean wooden cages (six animals per cage) and maintained at 25 ± 1°C with a relative humidity of 45-55%. They were allowed 12 h of light and dark cycles with free access to pelleted food from Topfeed® and water *ad libitum.* The animals were acclimatized to laboratory conditions for two weeks before the experiment began. Ethical approval was obtained from the Institutional Animal Ethical Committee and approved by the Ethical Review Board of the Faculty of Life Sciences of the University of Benin (LS24001). All experiments were performed according to the CPCSEA Guidelines.

Acute toxicity study

Acute toxicity was determined using the modified Lorke's (1983) method, which was recently reported by Osigwe *et al.* (2025). This process comprised two stages. The first stage involved 12 rats randomly placed in four groups (A, B, C, and D) of three rats each. The animals were administered methanol leaf extract of *A. cordifolia* by gavage in single doses of 10 mg/kg (A), 100 mg/kg (B), and 1000 mg/kg (C) per body weight, and the fourth group received 1 mL of distilled water. The animals were monitored for changes in behavior or death within 24 h of administration, and since mortality and behavioral changes were not recorded, the second stage of the experiment was conducted. This involved three rats, with one rat per group and administration of 1600 mg/kg per body weight of the extract to the first rat, 2900 mg/kg per body weight to the second rat, and 5000 mg/kg per body weight to the third rat. Mortality and changes in animal behavior were observed and recorded.

In vivo anti-inflammatory evaluation

Male rats were randomly distributed into five (5) groups (n=4) with a total of twenty (20) rats. Group I served as the negative control and received 1 mL of distilled water. Group II was the positive control group that received 300 mg/kg (p. o.) of acetylsalicylic acid. Groups III, IV, and V received 200, 400, and 800 mg/kg of the extract by gavage, respectively. Inflammation was induced 30 min later by a single sub-planar injection (left hind paw) of 0.2 mL solution of freshly prepared 6% (w/v) carrageenan suspension prepared in normal saline via the modified method of Agbaje et al. (2008). The increases in paw diameter were recorded using Vernier caliper and per cent inhibition were calculated using the formula below

$$\% \text{ inhibition} = (C_t\text{-}C_o)_C - (C_t\text{-}C_o)_T / (C_t\text{-}C_o)_C$$

Where: C_t = Paw thickness at time t; C_o = Initial paw thickness; $(C_t\text{-}C_o)_c$ = Increase in paw thickness of the control group; $(C_t\text{-}C_o)_T$ = Increase in paw thickness of the treatment groups; $C_t\text{-}C_o$ = Increase in paw thickness in control/ Treatment C_C / C_T

Bacteria Collection and Preparation

Drug-resistant clinical bacterial isolates used in this study were obtained from the University of Benin Teaching Hospital and cultured overnight in nutrient broth by inoculation. The bacteria used in this study were *Staphylococcus aureus, Streptococcus mutans, Escherichia coli,* and *Pseudomonas aeruginosa*. These isolates were confirmed through susceptibility testing with the standard testing with standard antibiotic gentamicin using the method described by the Clinical and Laboratory Standards Institute (CLSI, 2016). The organisms were standardized by preparing a suspension in 1 mL of normal saline or sterile water. The turbidity of the suspension was subsequently adjusted to match the 0.5 McFarland standard before evenly seeding the entire surface of the agar. These set-ups were carefully incubated overnight in an oven temperature of 37^0C overnight for uniformity of bacteria growth.

Preparation of Paper-Extract Disc

A paper disc diffusion experiment was used to confirm the efficacy of the extract on the aforementioned bacterial isolates. A sterile filter paper disc of 6 mm size was obtained from the whole filter paper, which was cut using a file punching machine. The cut paper size was placed in a container and sterilized by autoclaving at 121^0C for 15 min. Different concentrations of the extract were prepared in stoichiometric ratios by dissolving 1 g of the extract in 1 mL of sterile distilled water, from which other concentrations were obtained. Extract concentrations of 200, 100, 50, 25, and 12.5 mg/mL were prepared in separate sample bottles. Sterile paper discs were placed in the bottles and allowed to stand for 5 h before the antibacterial activity was assessed, although only 12.50 mg/mL of the prepared disc was utilized for this study.

Antibacterial Susceptibility Testing

The antibacterial activity was determined using standardized bacterial cells, which were streaked on Mueller Hinton agar (MHA) plates, before the discs of 12.5 mg/mL the extract was impregnated on the agar with extreme care with the aid of sterile forceps. The plates were well labeled and incubated for 24 h to monitor and measure any zone of inhibition that may occur. Standard discs with gentamicin (Oxoid, UK) were used in this study. The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the CLSI (2020) standard after incubation for 24 h at 37°C.

Statistical analysis

Data are presented as the average ± standard error of the mean and were analyzed using two-way analysis of variance, and post hoc analysis was performed using Tukey's analysis. The level of significance was set at $P < 0.05$ (a) and 0.01 (b).

Results

Yield of extract

The dried pulverized leaves of *A. cordifolia* (200 g) were macerated with 1.2 L of methanol for three days. The extract obtained had a percentage yield of 19.03%.

Phytochemical screening

The phytochemical constituents detected in the extract included alkaloids, tannins, triterpenoids, saponins, glycosides and flavonoids, whereas phytosterols and phenolic compounds were absent (Table 1)

Table 1: Phytochemical constituents of *A. cordifolia* leaf

Phytochemical constituents	Inference
Glycosides	+
Saponins	+
Flavonoids	+
Phenolics	-
Tannins	+
Phytosterols	-
Terpenoids	+
Alkaloids	+

Key: - = absent, + = present

Proximate Composition of *Alchornea cordifolia* Leaf

The proximate assay provides data of the primary metabolites and minerals which included carbohydrate (36.87%), protein (13.30%), fat (13.20%), fibre (17.00%), ash (4.32%) and moisture (15.40%) (Table 2)

Table 2: Proximate composition of *A. cordifolia* leaf

Components	Value (%)
Moisture	15.40±0.01
Ash	4.23±0.03
Crude fibre	17.00±0.02
Crude fat	13.20±0.02
Crude protein	13.30±0.02
Carbohydrate	36.87±0.01

Quantitative Elemental Analysis

Elemental analysis of the powdered leaves of *A. cordifolia* afforded high level of potassium (K); 37.40 mg/kg, while other elements detected were low; Zinc (Zn) (0.21 mg/kg), magnesium (Mg) (0.87 mg/kg), sodium (Na) (0.20 mg/kg), iron (Fe) (0.90 mg/kg), and copper (Cu) (0.10 mg/kg) (Table 3).

Table 3: Elemental contents of *Alchornea cordifolia* leaf extract

Mineral elements	Amount (mg/kg)
Zinc (Zn)	0.21
Magnesium (Mg)	0.87
Sodium (Na)	0.20
Iron (Fe)	0.90
Copper (Cu)	0.10
Potassium (K)	37.40

Acute toxicity

Oral administration of the methanol extract of *A. cordifolia* leaf up to 5000 mg/Kg did not cause any sign of toxicity or death suggesting that the LD_{50} is above 5000 mg/Kg.

Effects of A. cordifolia leaf extract on carrageenan-induced paw edema

Treatment with the extract *A. cordifolia leaf* elicited non-significant decrease ($P > 0.05$) in paw volume (Table 4)

Table 4: Effect of *A. cordifolia* leaf extract on paw size (in mm) edema in Wistar rats

Groups	Doses (mg/kg)	Paw-edema (mm)				
		30 min	60 min	120 min	180 min	240 min
Distilled water	0.5	2.90 ±0.05	2.90 ±0.00	2.90 ±0.00	2.90 ±0.03	2.90 ±0.05
Acetylsalicylic acid	300	4.33±0.12	4.23±0.13	3.60 ± 0.06	3.33±0.09	3.20±0.06
A. cordifolia extract	200	4.03±0.03	4.53±0.26	4.67±0.18	4.70±0.31	4.07±0.03
A. cordifolia extract	400	4.33±0.48	4.83±0.27	4.53±0.09	4.30±0.06	4.13±0.15
A. cordifolia extract	800	4.20±0.85	4.37±0.44	4.23±0.35	4.10±0.40	3.83±0.38

Antimicrobial effect of A. cordifolia leaf extract

After 24 h of incubation, the extract (12.50 mg/ml) inhibited the growth of *Pseudomonas aeruginosa*, while other bacterial species were resistant at this concentration. Gentamycin inhibited the growth of the bacteria used in this study with zones of inhibition range of 18 mm to 23.50 mm (Table 5).

Table 5: Zones of inhibition in millimeter of 12.5 mg/mL of *A. cordifolia* leaf extract

Bacteria	A. *cordifolia* extract (12.50 mg/mL)	Gentamycin
E. coli	-	23.50±0.5
P. aeruginosa	13.00±0.5	19.00±0.3
S. mutans	-	18.00±0.5
S. aureus	-	18.00±0.3

Discussion

Phytochemical screening of the ethanol leaf extract *A. cordifolia* previously revealed the presence of glycosides, saponins, flavonoids, and tannins (George *et al*., 2010), while the aqueous decoction contained tannins, glycosides, and flavonoids (Adounkpa *et al*., 2022). Alkaloids were present in our assay while the same was absent in both studies previously reported. These variations could be due to the different sample collection sites and extractive solvents used. Thus, the pharmacological effects of *A. cordifolia* may be ascribed to the presence of oleic acid, octe-3-ol, gallic acid, hypericin, yohimbine, alchorneine, methyl salicylate, citronellol, and 1,8-cineole (Sinan *et al*., 2021).

Analysis of the stability and primary metabolites in the leaves of *A. cordifolia* revealed high moisture levels, showing that the powdered drug retained some quantity of water that could encourage the growth of microbes. High levels of protein, fat, carbohydrates, and fiber validate its use as an edible food source (Foster *et al*., 2009). This result slightly contrasts with the values from Dowe et al. (2016), who reported higher values of fiber (65%) and carbohydrate (40%) for Massularia *acuminate.*

Minerals in crude drugs have been shown to have medicinal value. Minerals, such as zinc, magnesium, iron, copper, sodium, and potassium, have immune-boosting and wound-healing effects, enhance physical growth (Bhowmik *et al*., 2010), regenerate tissues and organs (Fiorentini *et al*., 2021), and maintain electrolyte imbalance (Yan *et al*., 2016). Ejeh *et al* (2023) analyzed the powdered leaf of *A. cordifolia* for mineral elements and observed high level of minerals such as zinc, magnesium, sodium, iron, copper.

The study of the extract in rats offered insights into its safety profile. The absence of obvious toxicity and mortality underscores the promising safety profile of *A. cordifolia* extract, providing reassurance for its potential use in medicinal applications. Gouch *et al*. (2021) previously reported that the LD_{50} of the aqueous extract of *A. cordifolia* leaves were 8000 mg/kg. Current anti-inflammatory drugs used to treat inflammation are associated with numerous adverse effects (Scheiman, 2016). Therefore, the exploration of better anti-inflammatory agents has become a significant research focus in recent years. Inflammation is crucial in the initiation and progression of various diseases; hence, targeting the inflammatory response could lead to effective treatment outcomes. Several inflammatory mediators, including PGE2, TNF-α, and IL-6, contribute to the development of inflammatory diseases (Hu *et al.,* 2019). Therefore, the inhibition of these pro-inflammatory mediators could potentially halt the onset and progression of inflammatory disease. The results obtained from this study demonstrated that the *A. cordifolia* extract did not significantly reduce paw edema in rats. Phytoconstituents, especially flavonoids, have been reported to attenuate inflammation (Gunathilake *et al.,* 2018; Mukhija and Sundriyal, 2013; Ahmadiani *et al*., 2000). The maximal percentage inhibition (35.40%) was observed at the 4th hour at a dose of 800 mg/kg. The anti-inflammatory activity exhibited by the *A. cordifolia* extract agrees with the report of Arega *et al.* (2023), who showed that *Premna schimperi* leaf extract reduced paw edema in acute inflammation models.

The methanol extract exhibited inhibitory action against *P. aeruginosa* compared to gentamicin. No activity was observed against *E. coli* or *S. mutans* at the tested concentrations. This result agrees with that of Agboke *et al*. (2020), who demonstrated that both aqueous and ethanol extracts of *A. cordifolia* leaves and stem bark were effective against *P. aeruginosa*, with MIC values ranging from 1.95 to 15.63 mg/mL. Owoseni *et al*. (2015) also noted that aqueous extracts of *A. cordifolia* had no inhibitory effect on *E. coli*. There is limited research on the activity of *A. cordifolia* against *S. mutans*. However, the general trend indicates that *A. cordifolia* exhibits more pronounced activity against Gram-negative bacteria like *P. aeruginosa* and *E. coli*, and Gram-positive bacteria such as *Staphylococcus aureus*, than against oral pathogens like *S. mutans*. The variations observed in the antimicrobial activity may be due to extraction solvent, part of plant used, geographical location and bacterial strain variability.

Conclusions

The results of this study indicate that the methanol extract of *Alchornea cordifolia* leaves may possess therapeutic potential owing to the presence of bioactive compounds such as glycosides, saponins, flavonoids, tannins, terpenoids, and alkaloids. This study also highlighted the

anti-inflammatory and antibacterial potential of *A. cordifolia,* especially against *P. aeruginosa*, implying that the extract can be used in the treatment of infections caused by these bacteria.

Conflict of interest

The authors declare no conflicts of interest before, during, or after the study.

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